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INSTRUCTION MANUAL

Quick-RNA™ Microprep Kit

Catalog Nos. R1050 & R1051

Highlights

- High-quality total RNA (including small RNAs) from a wide range of samples – single to 10⁶ cells.
- Isolate small and large RNAs into separate fractions (optional).
- *DNA-free* RNA for use in any downstream application. *DNase I included.*

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please contact us.

Some difficult-to-lyse samples may require mechanical or enzymatic homogenization. For assistance, contact us at tech@zymoresearch.com.

For 10^2 to 10^7 cells, use the **Quick-RNA™ Miniprep Kit** (Cat. Nos. R1054, R1055).

Product Contents

Quick-RNA™ Microprep Kit (Kit Size)	R1050 (50 Preps.)	R1051 (200 Preps.)
RNA Lysis Buffer	50 ml	2x 100 ml
RNA Prep Buffer	25 ml	100 ml
RNA Wash Buffer¹ (concentrate)	24 ml	2x 48 ml
DNase/RNase-Free Water	4 ml	10 ml
DNase I² (lyophilized)	1	4
DNA Digestion Buffer	4 ml	16 ml
Zymo-Spin™ IC Columns	50	200
Collection Tubes	50	200
Instruction Manual	1	1

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

Storage Temperature - Store all kit components (*i.e.*, buffers, columns) at room temperature. Store reconstituted DNase I at -20 °C.

¹ Before use, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate.

² Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

Specifications

- **Sample Sources** – Cells or tissue samples, yeast, plant or bacteria. Compatible with DNA/RNA Shield™ and RNAlater™.
- **Sample Storage** – Samples homogenized in RNA Lysis Buffer are stable and can be stored frozen prior to purification.
- **Sample Size** – Up to 10^6 cells or 5 mg tissue.
- **RNA Purity** – High quality RNA ($A_{260}/A_{280} >1.8$, $A_{260}/A_{230} >1.8$) suitable for all downstream RNA-based manipulations.
- **RNA Recovery** – Up to 10 µg RNA can be eluted into ≥ 6 µl RNase-free water allowing for a highly concentrated sample.
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored frozen. RNase inhibitors can be included for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility. RNAlater™ is a trademark of Ambion, Inc., Austin, Texas and is protected by various U.S. and foreign patents.

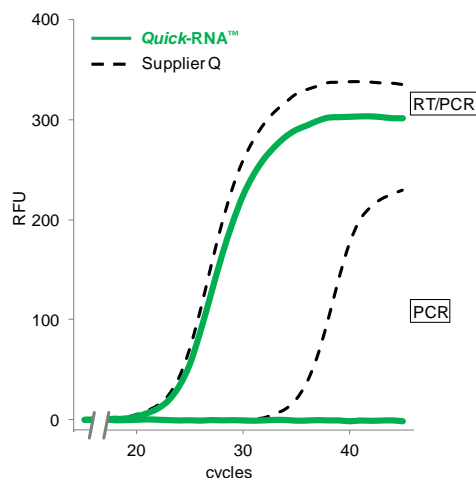
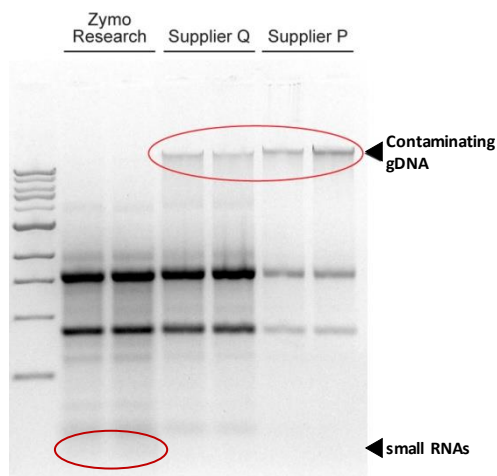
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Product Description

The **Quick-RNA™ Microprep Kit** is an innovative product designed for the easy, reliable, and rapid isolation of DNA-free RNA from a wide range of cell (*up to 10⁶*) and tissue samples (*up to 5 mg*). The procedure combines a unique buffer system with Clean-Spin™ column technology to yield high quality total RNA (*including small RNAs 17-200 nt*) in about 10 minutes.

The procedure is simple: Add the provided **RNA Lysis Buffer** to a sample, then purify the RNA using the **Zymo-Spin™ Columns**. The result is highly-concentrated, *DNA-free* RNA that is suitable for subsequent RNA-based methods including RT-PCR, hybridization, sequencing *etc.* In addition, the kit can be used for enrichment of small and large RNAs in two separate fractions (page 5).



The **Quick-RNA™ Microprep Kit** yields high quality total RNA. High levels of genomic DNA contamination are present in the preps from Suppliers Q & P but not with the **Quick-RNA™ Microprep Kit**. Total RNA was isolated from human epithelial cells (sans DNase treatment).

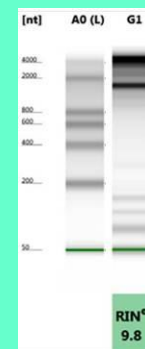
RNA isolated with the **Quick-RNA™ Microprep Kit** is DNA-free. Samples isolated with Supplier Q's kit are provided for comparison. Total RNA was isolated from 10⁶ human epithelial cells (with in-column DNase treatments for both kits). Each amplification curve represents an average of three independent isolation experiments.

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Notes:

Use the **Direct-zol™ RNA Miniprep Kit** (Cat. Nos. R2050, R2051, R2052, R2053) for isolation of RNA *directly* (without phase separation) from samples in Trizol®, *etc.*

Use the **DNA/RNA Shield™** for safe sample storage and transport at ambient temperatures.



The **Quick-RNA™** kits yield high quality RNA with high "RNA Integrity Numbers" (2200 TapeStation, Agilent).

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Ensure the RNA isolation procedure is performed in an RNase-free environment.

Notes:

Samples homogenized in **RNA Lysis Buffer** can be stored frozen for processing at a later time.

ZR Bashing Bead™ Lysis Tubes are available separately (Cat. Nos. S6002, S6003).

Processing plant tissue and other samples containing polyphenolics, humic acids, melanin, *etc.* may require use of the **OneStep™ PCR Inhibitor Removal Kit** (Cat. No. D6030).

Use the **DNA/RNA Shield™** for safe sample storage and transport at ambient temperatures.

Buffer Preparation

- ✓ Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1050) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1051).
- ✓ Reconstitute the lyophilized **DNase I** as indicated on the vial prior to use and store aliquots at -20°C.

Protocols

The RNA isolation consists of three steps: (I) *Sample Lysis/Homogenization*, (II) *Sample Clearing* and (III) *RNA Purification*.

All steps should be performed at room temperature (20-30 °C).

I. Sample Lysis/Homogenization

Recommended **RNA Lysis Buffer** volumes

RNA Lysis Buffer	100 µl	300 µl
Cells	Up to 10 ⁵	Up to 10 ⁶
Tissue	-	Up to 5 mg

Adherent Cells

Lyse cells directly in the culture container by removing liquid medium and adding **RNA Lysis Buffer** directly to the monolayer.

Cells in Suspension

Pellet cells ($\leq 500 \times g$), remove the supernatant completely then resuspend the cell pellet in **RNA Lysis Buffer**. Vortex briefly.

Tissue and Tough-to-Lyse Samples

Fresh or frozen tissue (animal, plant, insect, yeast or bacteria) can be mechanically homogenized (e.g., **ZR BashingBead™ Lysis Tubes**) directly in the **RNA Lysis Buffer**.

Alternatively, tough-to-lyse tissue samples can be Proteinase K treated (page 5).

Liquids/Reaction Clean-up

DNase-treated RNA, labeling and *in vitro* transcription reactions can be processed directly by adding 4 volumes of **RNA Lysis Buffer** to each volume of sample (4:1) then mixing well.

Samples in DNA/RNA Shield™

Bring samples homogenized and stored in **DNA/RNA Shield™** to room temperature (20-30 °C). Then add 1 volume **RNA Lysis Buffer** (1:1), mix and proceed with Sample Clearing step.

Samples in DNA/RNA Shield™ can be Proteinase K treated (page 5).

Samples in RNA/later™

To process cells or liquids in **RNA/later™** (without reagent removal): Add 1 volume of RNase-free water or PBS to the sample (1:1). Then add 4 volumes **RNA Lysis Buffer** (4:1) and mix.

Alternatively, remove the **RNA/later™**, then proceed with Sample Lysis/Homogenization according to the sample type.

II. Sample Clearing

The following is recommended for cells and tissue (animal/plant) but can be omitted for cell-free liquids and low input samples ($\leq 10^5$ cells).

For particulate removal, centrifuge lysates at $\geq 12,000 \times g$ for 1 minute. Then transfer the supernatant into an RNase-free tube (*not provided*).

III. RNA Purification

All centrifugation steps should be performed at 10,000-16,000 $\times g$.

1. Add 1 volume ethanol (95-100%) to the sample in **RNA Lysis Buffer** (1:1). Mix well.
2. Transfer the mixture to a **Zymo-Spin™ IC Column¹** in a **Collection Tube** and centrifuge for 30 seconds. Discard the flow-through.
3. **In-column DNase I Treatment** (optional)

This step can be used for trace DNA removal.

- a. Prewash the column with 400 μ l **RNA Wash Buffer**. Centrifuge for 30 seconds. Discard the flow-through.
- b. For each sample to be treated, prepare **DNase I Reaction Mix** in an RNase-free tube (*not provided*). Mix well by gentle inversion:

DNase I²	5 μ l
DNA Digestion Buffer	35 μ l

- c. Add 40 μ l **DNase I Reaction Mix** directly to the column matrix. Incubate at room temperature (20-30 °C) for 15 minutes.

4. Add 400 μ l **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
5. Add 700 μ l **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
6. Add 400 μ l **RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (*not provided*).
7. Add 15 μ l **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

Alternatively, for highly concentrated RNA use ≥ 6 μ l elution.

The eluted RNA can be used immediately or stored at -70°C.

Notes:

¹ To process samples >700 μ l, **Zymo-Spin™** columns may be reloaded.

² Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

Unit definition - one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.

Purification of Small and Large RNAs into Separate Fractions

This procedure is compatible with animal cell inputs (up to 10⁶) or previously isolated RNA only.

All centrifugation steps should be performed between 10,000-16,000 x g.
This protocol requires two columns (per prep).

Notes:

¹ Adjust the sample volume to 50 µl (minimum).

² **Zymo-Spin™** columns may be reloaded to process samples >700 µl.

- Mix an equal volume of **RNA Lysis Buffer** and ethanol (95-100%).
Example: Mix 50 µl buffer and 50 µl ethanol.
- Add 2 volumes of the buffer/ethanol to an RNA sample¹ or 300 µl buffer/ethanol to a cell pellet and mix.
Example: Mix 100 µl buffer/ethanol and 50 µl sample.
- Transfer the mixture² to the **Zymo-Spin™ Column** and centrifuge for 30 seconds. **Save the flow-through!**

Column: RNAs >200 nt

- Continue to step 5.

Flow-through: RNAs 17-200 nt

Add 1 volume ethanol and mix.

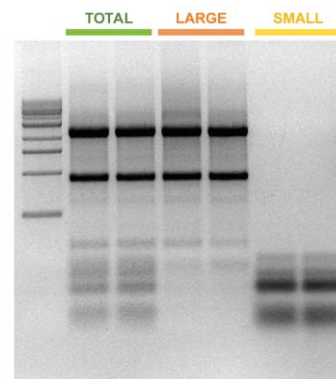
Example: Add 150 µl ethanol to 150 µl flow-through.

Transfer the mixture to a new column and centrifuge for 30 seconds. Discard the flow-through.

- Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
- Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
- Add 400 µl **RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).
- Add 15 µl **DNase/RNase-Free Water** directly to the column matrix, then centrifuge for 30 seconds.

Alternatively, for highly concentrated RNA use ≥6 µl elution.

The eluted RNA can be used immediately or stored at -70°C.



Total RNA (>17 nt), large (>200 nt) or small RNAs (17-200 nt) are effectively partitioned and purified with the **Quick-RNA™** kit.

³ **2X Digestion Buffer** (Cat. No. D3050-1-5 and D3050-1-20).

⁴ **Proteinase K** (Cat. No. D3001-2-5 and D3001-2-20).

One unit of enzyme will hydrolyze urea-denatured hemoglobin to produce 1.0 µmole of tyrosine per minute at pH 7.5 at 37°C.

Proteinase K Digestion

Example: up to 5 mg solid tissue or 10⁶ animal cells in DNA/RNA Shield™
2X Digestion Buffer³
Proteinase K⁴

95 µl
95 µl
≥6 U

Prepare a Proteinase K reaction mix (see example above, scale-up as necessary). Incubate at 55°C for 30 minutes (e.g., pelleted white blood cells) or 1-3 hours (solid tissue). Then add 1 volume **RNA Lysis Buffer** and proceed to Sample Clearing (page 4).

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Ordering Information

Product Description	Input	Binding	Catalog No.	Kit Size
Quick-RNA™ Microprep Kit	~1-10 ⁶ cells	~10 µg	R1050	50 Preps.
			R1051	200 Preps.
Quick-RNA™ Miniprep Kit	~10 ² -10 ⁷ cells	~100 µg	R1054	50 Preps.
			R1055	200 Preps.
			R1057T	10 Preps.
Quick-RNA™ Miniprep Plus Kit	~10 ² -10 ⁷ cells	~100 µg	R1057	50 Preps.
			R1058	200 Preps.
Quick-RNA™ Midiprep Kit	~10 ⁶ -10 ⁸ cells	~1 mg	R1056	25 Preps.
Quick-RNA™ 96 Kit	~1-10 ⁶ cells	~10 µg/well	R1052	2x 96 Preps.
			R1053	4x 96 Preps.

For Individual Sale	Catalog No.	Amount
RNA Lysis Buffer	R1060-1-50	50 ml
	R1060-1-100	100 ml
RNA Prep Buffer	R1060-2-10	10 ml
	R1060-2-25	25 ml
	R1060-2-100	100 ml
RNA Wash Buffer (concentrate)	R1003-3-6	6 ml
	R1003-3-12	12 ml
	R1003-3-24	24 ml
	R1003-3-48	48 ml
DNase I (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
Zymo-Spin™ IC Column	C1004-50	50
	C1004-250	250
	C1001-50	50
Collection Tube	C1001-500	500
	C1001-1000	1000
	W1001-1	1 ml
DNase/RNase-Free Water	W1001-6	6 ml
	W1001-10	10 ml

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